Available online at http://www.journalijdr.com

IJDR

International Journal of DEVELOPMENT RESEARCH

ISSN: 2230-9926

International Journal of Development Research
Vol.1, Issue, 2, pp.006-011, March, 2011

Full Length Research Article

EFFECTS OF CADMIUM, COPPER, LEAD AND ZINC ON BIOMARKER RESPONSES IN POST LARVAL STAGES OF PENAEUS MONDON

Rajkumar, J. S. I[1]*, John Milton, M. C[1] and Arockia Rita, J.J[2]

[1]Department of Advanced Zoology and Biotechnology, Loyola College, Chennai 600 034, Tamil Nadu, India.
[2]Department of Zoology, Quaid E Millath College (Govt.), Chennai – 600 002, Tamil Nadu, India.

ARTICLE INFO

Article History:
Received 22nd January, 2010
Received in revised form
25th February, 2011
Accepted 21st March, 2011
Published online 25th April, 2011

Key words:
Reactive oxygen species, reduced glutathione, catalase, glutathione S-transferase, lipid peroxidation, *Penaeus monodon* and Oxidative stress.

ABSTRACT

In the present investigation, cadmium, copper, lead and zinc induced biochemical changes were studied in post larval stages of *Penaeus monodon* under long term chronic test (30 days). The total protein content, level of lipid peroxidation, reduced glutathione and the activity of catalase, glutathione *S*-transferase and acetylcholinesterase in the tissues of *Penaeus monodon* decreased with the increase in concentrations of heavy metal concentrations used in the long term chronic test for 30 days. Enzymatic antioxidants have great potential to indicate the toxic effects of heavy metals because they can generate free radicals inducing lipid peroxidation of cell membranes which was observed in the present study. The metal-mediated production of ROS is detoxified by a set of antioxidant enzymes, which are of great diagnostic value for environmental pollution indices and have proved the ability of being a biomarker in the present study with *Penaeus monodon.*

© Copy Right, IJDR, 2011, Academic Journals. All rights reserved.

INTRODUCTION

Heavy metals deplete glutathione and protein bound sulfhydryl groups, resulting in enhanced production of Reactive Oxygen Species (ROS) such as superoxide anion, hydrogen peroxide and hydroxyl radicals. The sequential reduction of oxygen leads to generation of superoxide anion and hydrogen peroxide (Monferran *et al.,* 2008). Superoxide anion also rapidly reacts with nitric oxide, yielding yet another reactive species peroxynitrite. All of these ROS have the potential to trigger cellular death. ROS are considered as crucial mediators for the metal-triggered tissue injuries and apoptosis (Liu *et al.,* 2009). To prevent oxidation induced damage, there must be effective antioxidation systems in the organisms. Some components of these systems involve reduced glutathione (GSH) and certain antioxidant enzymes including free radical scavenging enzymes, such as Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidases (GPX) and Glutathione Reductase (GR). Other associated enzymes are the Glyoxalase I (GI), Glyoxalase II (GII) and Glutathione S-Transferase (GST). Under oxidative stress conditions, ROS can be reduced by GSH, with the concomitant formation of the oxidized disulphide, oxidized glutathione (GSSG) (Lushchak and Bagnyukova, 2007).

***Corresponding author:** rajloyola83@gmail.com

A radical attack on lipids leads to the formation of lipid peroxides, which can decompose to yield alkanes, ketones and aldehydes. The variety of Lipid Peroxidation (LPO) by-products can also exert adverse biological effects in exposed organisms (Zielinski and Portner, 2000). The quantification of the diverse products of peroxidation is now being exploited as biomarker of oxidative stress. Intracellular reactions involved in the metabolism of heavy metals can increase the concentrations of superoxide anion, hydrogen peroxide and hydroxyl radical (Shi *et al.,* 2004). GSH is normally present in tissues at relatively high concentrations and is a key component of antioxidant defense mechanisms. Determinations of sulfhydryl group levels which metals have high affinity towards, and total protein levels could be beneficial in estimating the toxicity of metals (Gravato *et al.,* 2006). The antioxidant defense system is being increasingly studied because of its potential utility to provide biochemical biomarkers that could be used in environmental monitoring system (Oliveira *et al.,* 2010). Biomarkers in environmental monitoring confer significant advantages over traditional chemical measurements because measured biological effects can be meaningfully linked to environmental consequences so that environmental concerns can be directly addressed. Although the activity of antioxidant enzymes may be increased or inhibited under chemical stress, there is, however, no general rule for the different enzymes (Cheung *et al.,* 2001). The antioxidant enzymes tend to respond differently to

different chemical compounds; therefore, the activity of an individual antioxidant enzyme cannot serve as a general marker of oxidative damage. As a result, multiple antioxidant values are often measured together to indicate the total oxyradical scavenging capacity and this has been observed to provide greater indicating value (Regoli *et al.,* 2002). Changes in the activity of enzymes and other biomarkers are the possible tools for aquatic toxicological research. Although physical and chemical parameters are essential for risk determination, during the past decade the results of biological response to chemical stress have been used as references to determine the expected biological damage (Chapman and Wang, 2001).

MATERIALS AND METHODS

Post larval stages of *Penaeus monodon* were immediately transported to the laboratory in air filled plastic bags and acclimatized in glass aquaria with aerated natural filtered seawater for a period of 8 days at 28 PSU salinity, temperature of 28 ±2 °C, dissolved oxygen of 6.1 mg/l and pH of 7.98. Stock solutions of cadmium, copper, lead and zinc were freshly prepared by dissolving the proper metal salts of cadmium chloride hemi (pentahydrate), copper (II) chloride, lead (II) nitrate, and zinc sulfate in deionized (double distilled) water. Fresh stock solutions were prepared daily. These solutions were serially diluted to get the experimental concentration for the toxicity test. The experimental method includes static renewal (24 hour renewal) test. Five concentrations in a geometric series including control were prepared for the test for 30 days in chronic toxicity test (Table 1). Toxicant and seawater were replaced on daily basis. Test animals were fed three times during the test. Maximum-allowable control mortality was 20 per cent for 30 days for chronic (USEPA, 2002a & b).

Sample Preparation

At the final stages of the chronic toxicity test, the tissue samples of survived test animals were pooled and made in duplicates. For the analysis of lipid peroxidation marker and antioxidant enzyme activities, 1g tissue was homogenized in chilled pestle and mortar with 5ml homogenization buffer (0.25Msucrose, 10 mMTris, 1 mMEDTA, and pH 7.4) and centrifuged at 5,000 rpm for 15 minutes at 4°C. The resulting supernatant was the homogenate which was used for the estimation of various biochemical assays.

Lipid Peroxidation (LPO)

Lipid peroxidation level was assayed by measuring Malondialdehyde (MDA), a decomposed product of polyunsaturated fatty acids. Hydroperoxides were determined by the thiobarbituric acid reaction as described by Ohkawa *et al.* (1979) and was measured at 532 nm in the UV-Spectrophotometer. The absorbance was read at 532 nm after removal of any flocculated material by centrifugation. The amount of Thiobarbituric Acid Reactive Substance (TBARS) was calculated by using an extinction coefficient of 1.56 x 105/M/cm and expressed as nmol TBARS formed /mg protein.

Glutathione S-Transferase (GST)

Activity of Glutathione *S*-transferase (GST) was assayed at 340 nm by measuring the increase in absorbance using 1-chloro-2, 4-dinitrobenzene (CDNB) as the substrate according to the method followed by Habig *et al.* (1974). The results were expressed as nM of GSH and CDNB conjugate formed /min/mg protein.

Catalase (CAT)

Catalase (CAT) activity was measured at 240 nm by determining the decay of hydrogen peroxide levels followed by Beers and Seizer (1952) and was expressed as µmol of hydrogen peroxide consumed /min/mg/protein.

Reduced Glutathione (GSH)

The reduced glutathione (GSH) was measured at 412 nm using 5, 5-dithiobis-(2-nitrobenzoic acid) (DTNB) reagent by the method of Moron *et al.* (1979). The values were expressed as µmol of GSH oxidized/mg protein.

Acetylcholinesterase activity (AChE)

Acetylcholine esterase activity (AChE) activity was determined using Ellman's reagent, DTNB (5, 5'-dithio-bis (2-nitrobenzoic acid); 0.5mM) and acetylthiocholine iodide (ACTI) as substrate (Alves *et al.,* 2002). The rate of change of absorbance at 412 nm was recorded over 1.5 minutes at 25°C. Blank samples were read to make sure that there was no non-specific esterase or other background activity and was expressed as nmol ACTI /min/mg/protein.

Total Protein

The protein concentration of each of the sample extract was determined according to Lowry (1951) using bovine serum albumin as the standard (mg protein/g tissue), the reaction mixture was measured at 750 nm.

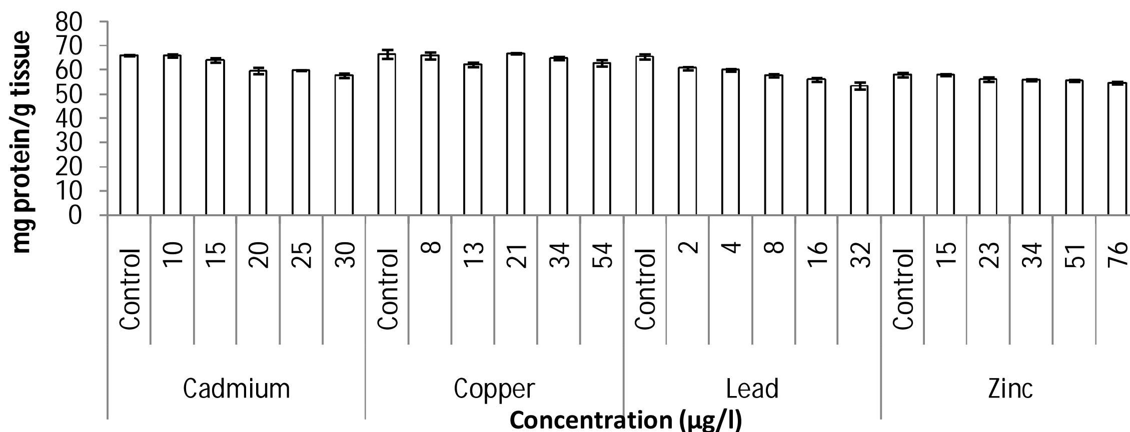
RESULTS

Total protein content, level of LPO, GSH, the activity of GST and CAT in the tissues of *P.monodon* showed significant (P<0.001) decrease with increase in the cadmium concentration (Figure 1, 2, 3, 4 and 6). The activity of AChE in the tissues of *P.monodon* had no significant variation except in 30 µg/l of significant (P<0.05) decreased activity of AChE (Figure 5). *P.monodon* exposed to copper in the short-term chronic toxicity test showed that total protein level and the activity of CAT had no significant variation in both the responses the effect was produced by 13 µg/l copper concentration significant at P<0.05 (Figure 1 and 4). Significant (P<0.001) increase in the activity of GST and level of GSH, LPO was observed when *P.monodon* was exposed to 21, 34 and 51 µg/l copper concentration (Figure 2, 3 and 6). The activity of AChE was significantly (P<0.001) decreased (Figure 5). The total level of protein, GST activity, level of GSH, AChE activity and level of LPO significantly (P<0.001) decreased along the increasing trend in the tissues of *P.monodon* exposed to lead concentration (Figure 1, 2, 3, 5 and 6). The activity of catalase was significantly (P<0.01and P<0.001) enhanced in the first three concentrations (Figure 4). The total protein level in *P.monodon* significantly decreased with increase in concentration of zinc (Figure 1). The activity of GST significantly (P<0.001) increased with increasing zinc

Table 1. Concentrations of cadmium, copper, lead and zinc used in the long term test for *P.monodon*

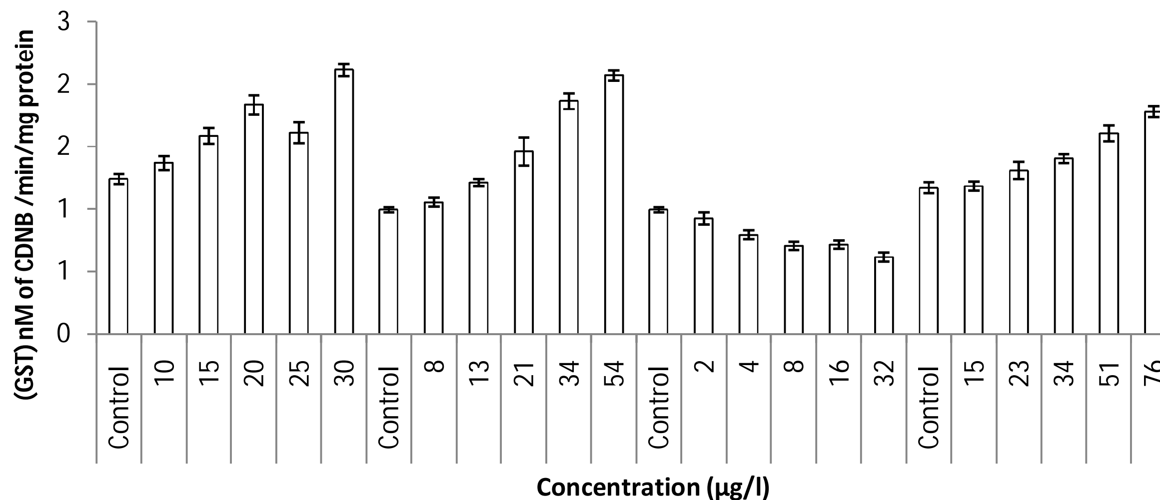
Cd Concentration (µg/l)	Cu Concentration (µg/l)	Pb Concentration (µg/l)	Zn Concentration (µg/l)
Control	Control	Control	Control
10	8	2	15
15	13	4	23
20	21	8	34
25	34	16	51
30	54	32	76

concentrations (Figure 2). The level of GSH in the tissues of *P.monodon* increased significantly (P<0.01) at 51 and 76 µg/l (Figure 3). The significant (P<0.05) increase in the level of GSH was also observed in the 23 and 34 µg/l of zinc concentration. Zinc concentration of 23 µg/l induced the effect of significant (P<0.05) increase in the activity of catalase (Figure 4). In the activity of AChE the highest concentration was found to have exerted a significant (P<0.05) effect on the AChE level in the tissues of *P.monodon* (Figure 5). Significant (P<0.05) increase in the level of LPO was observed when *P.monodon* was exposed to 51 and 76 µg/l (Figure 6).



**Values represented are the mean and standard deviation*

Fig. 1. Variation of total level of protein in *P.monodon* exposed to cadmium, copper, lead and zinc in short-term chronic toxicity test

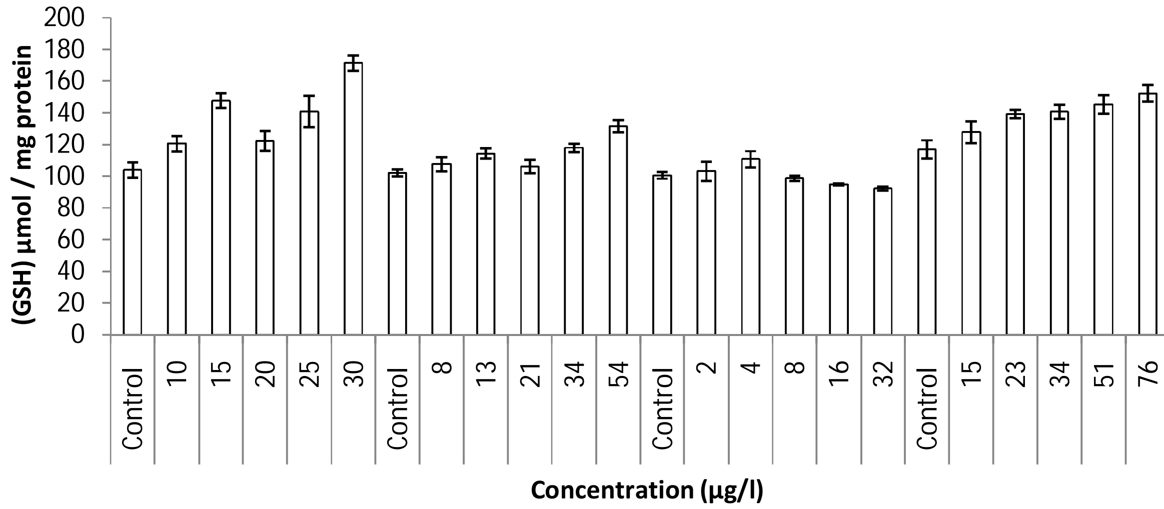


**Values represented are the mean and standard deviation*

Fig. 2. Variation of antioxidant enzyme (GST) in *P.monodon* exposed to cadmium, copper, lead and zinc in short-term chronic toxicity test

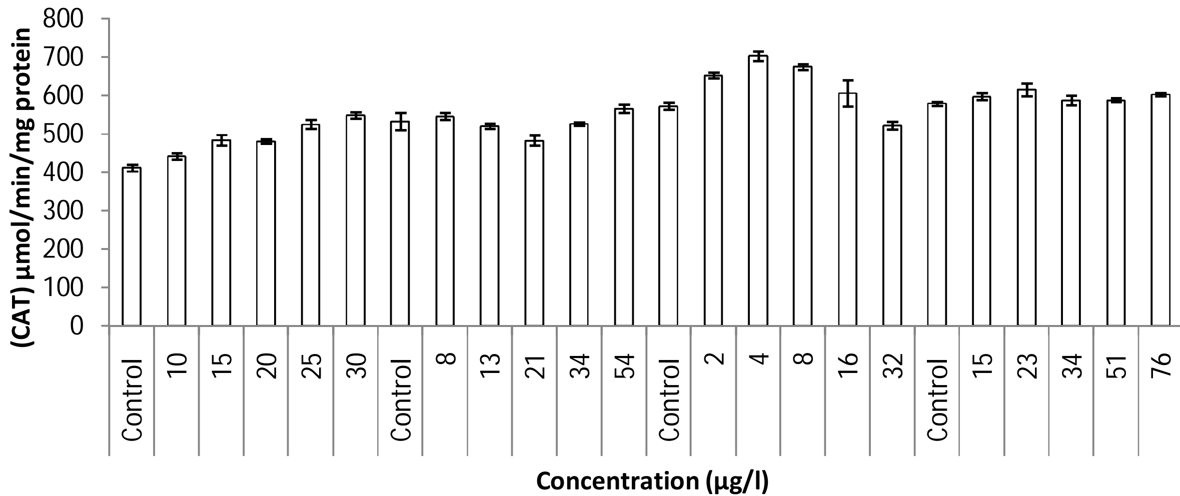
DISCUSSION

Aquatic organisms maintain high content of GSH in tissues and increased content has the function of protection (Thomas and Juedes, 1992). High content of GSH could be a consequence of its increased synthesis due to high cysteine accessibility, which is necessary for GSH synthesis. GSH content increased after treatment with cadmium (Son *et al.,* 2001). This could provide the first line of defense against the influence of toxic heavy metals. MacFarlane *et al.* (2006) found that heavy metals induce increase of GSH content in the crab *Parasesarma erythodactyla,* while decrease of GSH content in crab *Scylla serrata* was reported by Vijayavel and Balasubramanian (2006). It has been established that GSH content are related to the survival rate of mussels (Llopis *et al.,* 2002). Although pollution influence on GSH content is



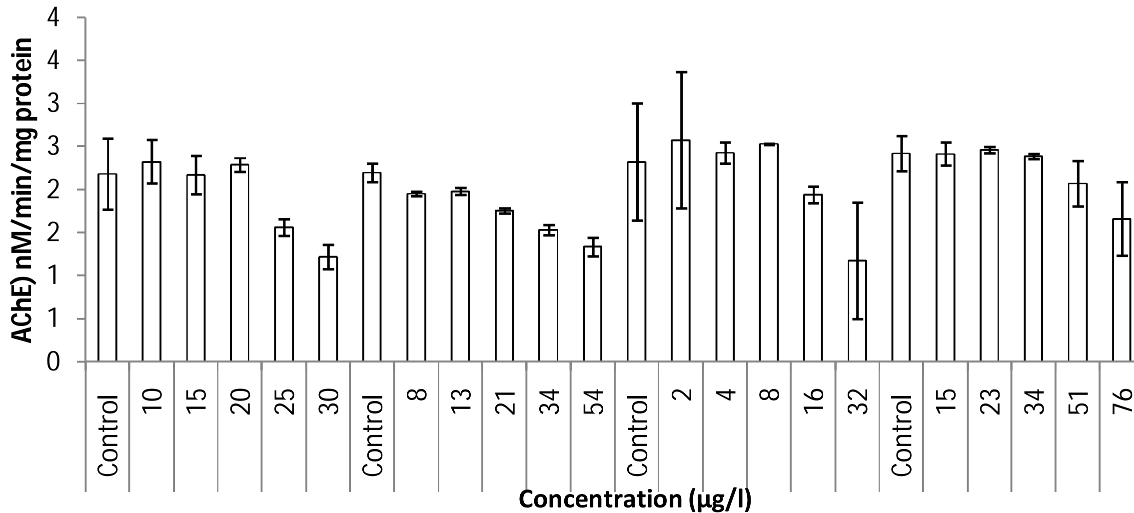
*Values represented are the mean and standard deviation

Fig. 3. Variation of GSH in *P.monodon* exposed to cadmium, copper, lead and zinc in short-term chronic toxicity test



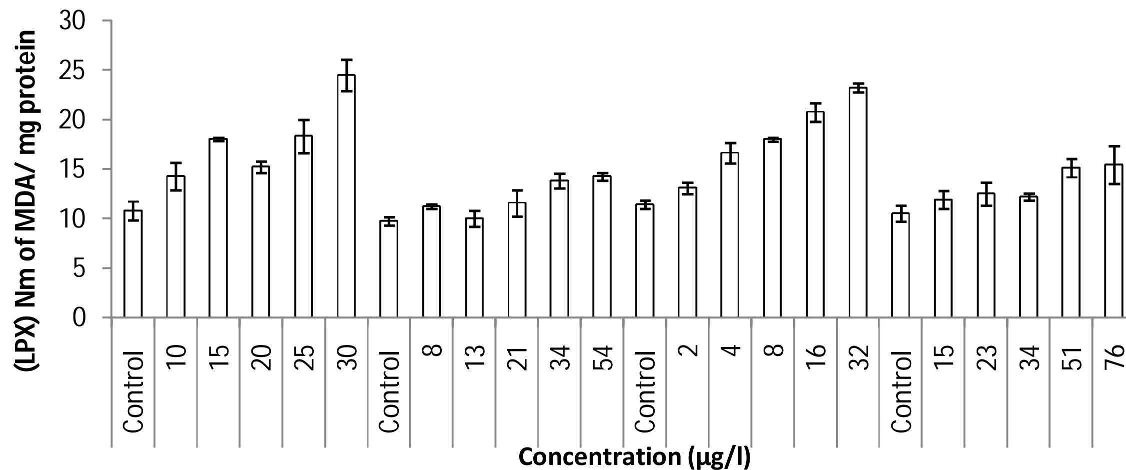
*Values represented are the mean and standard deviation

Fig. 4. Variation of catalase in *P.monodon* exposed to cadmium, copper, lead and zinc in short-term chronic toxicity test



*Values represented are the mean and standard deviation

Fig. 5. Variation of AChE in *P.monodon* exposed to cadmium, copper, lead



*Values represented are the mean and standard deviation

Fig. 6. Variation of lipid peroxidation in *P. monodon* exposed to cadmium, copper, lead and zinc in short-term chronic toxicity test

regulated by a feedback mechanism, the GSH levels can be used as a potential biomarker in fish (Oost *et al.,* 1996).The reduction in protein content might be also due to the proteolysis process for energy production and utilization owing to the decreased food intake of crabs under stress (Elumalai and Balasubramanian, 1999). The results obtained in our study show the existence changes in GSH content. These data may indicate a faster rate of GSH utilization or degradation, which could be responsible for the observed lower GSH content. Moreover, increase of GSH content may be related to prevention of oxidative challenge (Dandapat *et al.,* 2000). The removal of H_2O_2 is an important strategy of marine organisms against oxidative stress (Valavanidis *et al.,* 2006). Increased activities of CAT have been reported in several fish and invertebrate species (Stephensen *et al.,* 2000). CAT activity in mussels is not sufficient to eliminate H_2O_2 before the formation of hydroxyl radicals (Bebianno *et al.,* 2005). Concentration of LPO was significantly higher ($P< 0.05$) in higher concentrations of cadmium, copper, lead and zinc. CAT activity was reduced due to increased levels of exposure indicating the importance of antioxidant (Pampanin *et al.,* 2005).

Fridovich (1998) suggested that hydroxyl radicals (HO^-) was produced by reduction of H_2O_2 by copper. These radicals are extraordinarily powerful oxidants and attack most organic compounds at diffusion-limited rates (Suzuki *et al.,* 1996). Copper is known to induce severe oxidative stress and a toxic effect on the polychaetes (Geracitano *et al.,* 2004). Geracitano *et al.* (2004a) observed a differential toxic response to copper exposure by the polychaete *Laeonereis acuta*, varying from acute to chronic. Copper accumulation also plays a role in the apoptosis process (Santon *et al.,* 2003). The visible changes of morphology and movement in the copper-exposed polychaetes indicate cytotoxic effects. It may be that ROS might cause a toxic effect in *Perinereis nuntia* through processes such as LPO and apoptosis. The excess metal could then combine with metabolically available parts to cause toxic effects (Redeker *et al.,* 2006).

Conclusion

Certain biomarkers of oxidative stress were estimated in *Penaeus monodon* under long term test (30 days). The results indicate significant elevation of lipid peroxidation in the tissues of *Penaeus monodon* exposed to cadmium, copper, lead and zinc. Under acute oxidative stress, the toxic effect of the pollutants may overwhelm the antioxidant defense. Furthermore, the apparent decrease in detoxification system in the gills, the first point of contact with heavy metals indicates that this system is a sensitive biochemical indicator of environmental pollution in the *Penaeus monodon.* The concentrations are often higher than those found in the environment, when compared with the present study. Hence, there is still a need to increase our understanding of the molecular mechanisms involved in biomarker responses at environmentally relevant concentrations of heavy metals. In view of the state of pollution in marine environment it is indeed an urgent need of the hour to evaluate the ecotoxicological impact of various types of contaminants in the marine environment using molecular biomarkers.

REFERENCES

Alves, S. R., Severino, C. Ibbotson, P.C. Silva, D.P. Lopes, A.Z. Saenz, F.R.A.S and Bainy, L.A. 2002. Effects of furadan in the brown mussel *Perna perna* and in the mangrove oyster *Crassostrea rhizophorae*. *Mar. Environ. Res*., 54: 241-245.

Bebianno, M.J., Company, R. Serafim, A. Cosson, R.P. and Medoni, A.F. 2005. Antioxidant systems and lipid peroxidation in *Bathymodiolus azoricus* from Mid-Atlantic Ridge hydrothermal vent fields. *Aquat. Toxicol*., 75: 354-373.

Beers, R.F and Seizer, I.W. 1952. Spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J. Biolo. Chem*., 115: 133-140.

Chapman, P.M and Wang, F. 2001. Assessing sediment contamination in estuaries. *Environ. Toxicol. Chem*., 20: 3-22.

Cheung, C.C.C., Zheng, G.J. Li, A.M.Y. Richardson, B.J and Lam, P.K.S. 2001. Relationship between tissue concentrations of polycyclic aromatic hydrocarbons and antioxidative responses of marine mussels, *Perna viridis*. *Aquat. Toxicol*., 52: 189-203.

Dandapat, J., Chainy, G.B.N and Rao, J.K. 2000. Dietary vitamin-E modulates antioxidant defence system in giant freshwater prawn,

Macrobrachium rosenbergii. *Comp. Biochem. Physiol. Part C.* 127: 101-115.

Elumalai, M and Balasubramanian, M.P. 1999. Influence of naphthalene on esterase activity during vitellogenesis of marine edible crab, *Scylla serrata*. *Bull. Environ. Contam. Toxicol*., 62(6): 743-748.

Fridovich, I. 1998. Oxygen toxicity: A radical explanation. *J. Exp. Biol*., 201: 1203-1209.

Geracitano, L.A., Bocchetti, R. Monserrat, J.M. Regoli, F. and Bianchini, A. 2004. Oxidative stress responses in two populations of *Laeonereis acuta* (Polychaeta: Nereididae) after acute and chronic exposure to copper. *Mar. Environ. Res*., 58:1-17.

Gravato, C., Teles, M. Oliveira, M. and Santos, M.A. 2006. Oxidative stress, liver biotransformation and genotoxic effects induced by copper in *Anguilla anguilla* L.-the influence of pre-exposure to bnaphthoflavone. *Chemos*., 65: 1821-1830.

Habig, W.H., Papst M.J and Jacoby, W.B. 1974. Glutathione S-transferase, the first step in mercapturic acid formation. *J. Biolo. Chem*., 249: 7130-7139.

Liu, J., Qu, W and Kadiiska, M.B. 2009. Role of oxidative stress in cadmium toxicity and carcinogenesis. *Toxicol. Appl. Pharmacol*., 238: 209-214.

Llopis, P.S., Ferrando, M.D and Pena, J.B. 2002. Fish tolerance to organophosphate-induced oxidative stress is dependent on the glutathione metabolism and enhanced by N-acetylcisteine. *Chemos*., 45: 671-681.

Lowry, O.H., Rosebrough, N.J. Farr, A.L and Randall, R.J. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem*., 193: 265-275.

Lushchak, V.I and Bagnyukova, T.V. 2007. Hypoxia induces oxidative stress in tissues of a goby, the rotan *Perccottus glenii. Comp. Biochem. Physiol. Part B. Biochem. Mol. Biol*., 148(4): 390-397.

MacFarlane, G.R., Schreider, M and McLennam, B. 2006. Biomarkers of heavy metal contamination in the red-fingered marsh crab, *Parasesarma erythodactyla. Arch. Environ. Contam. Toxicol*., 51: 584-593.

Monferran, M.V., Pesce, S.F. Cazenave, J and Wunderlin, D.A. 2008. Detoxification and antioxidant responses in diverse organs of *Jenynsia multidentata* experimentally exposed to 1, 2- and 1, 4-dichlorobenzene. *Environ. Toxicol*., 23: 184-192.

Moron, M.S., Beperre, J.W and Wick, B. 1979. Levels of glutathione, glutathione reductase, glutathione-S transferase in rat lung and liver. *Biochem. Biophys. Acta*., 582: 67-78.

Ohkawa, H. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem*., 95: 351-358.

Oliveira, M., Ahmad, I. Maria, V.L. Pacheco M. and Santos, M.A. 2010. Monitoring pollution of coastal lagoon using Liza aurata kidney oxidative stress and genetic endpoints: An integrated biomarker approach. *Ecotoxicol*., 19: 643-653.

Oost, V.R., Goksoyr, A. Celander, M. Heida, H. and Vermeulen, N.P.E. 1996. Biomonitoring of aquatic pollution with feral eel (*Anguilla anguilla*). II Biomarkers: Pollution induced biochemical responses. *Aquat. Toxicol*., 36: 189-222.

Pampanin, D.M., Camus, L. Gomiero, A. Marangon, I. Volpato, E. and Nasci, C. 2005. Susceptibility to oxidative stress of mussels (*Mytilus galloprovincialis*) in the Venice Lagoon (Italy). *Mar. Pollut. Bull*., 50: 1548-1557.

Redeker, E.S., Campenhout, K.V. Bervoets, L. Reijnders, H and Blust, R. 2006. Subcellular distribution of Cd in the aquatic Oligochaete *Tubifex tubifex*, implications for trophic availability and toxicity. *Environ. Poll*., 148: 166-175.

Regoli, F., Pellegrini, D. Winston, G.W. Gorbi, S. Giuliani, S. Lamberti, C.V and Bompadre, S. 2002. Application of biomarkers for assessing the biological impact of dredged materials in the Mediterranean: The relationship between antioxidant responses and susceptibility to oxidative stress in the red mullet (*Mullus barbatus*). *Mar. Poll. Bull*., 44: 912-922.

Santon, A., Irato, P. Medici, V. D'Inca, R. Albergoni, V and Sturniolo, G.C. 2003. Effect and possible role of Zn treatment in LEC rats, an animal model of Wilson's disease. *Biochim. Biophys. Acta*., 1637: 91-97.

Shi, H., Hudson, L.G and Liu, K. 2004. Oxidative stress and apoptosis in metal ion-induced carcinogenesis. *Free Radic. Biol. Med*., 37: 582-593.

Son, M.H., Kang, K.W. Lee, C.H and Kim, S.G. 2001. Potentiation of cadmium-induced cytotoxicity by sulfur amino acid deprivation through activation of extracellular signal-regulated kinase1/2 (ERK1/2) in conjunction with p38 kinase or c-Jun N-terminal kinase (JNK): Complete inhibition of the potentiated toxicity by U0126 an ERK1/2 and p38 kinase inhibitor. *Biochem. Pharmacol*., 62: 1379-1390.

Stephensen, E., Svavarsson, J. Sturve, J. Ericson, G. Erici, M.A. and Forlin, L. 2000. Biochemical indicators of pollution exposure in shorthorn sculpin (*Myoxocephalus scorpius*), caught in four harbours on the southwest coast of Iceland. *Aquat. Toxicol*., 48: 431-442.

Suzuki, K.T., Rui, M. Ueda, J.I and Ozawa, T. 1996. Production of hydroxyl radicals by copper containing metallothionein: Roles as prooxidant. *Toxicol. Appl. Pharmacol*., 141: 231-237.

Thomas, P and Juedes M.J. 1992. Influence of lead on glutathione status of Atlantic croaker tissues. *Aquat. Toxicol*., 23: 11-30.

USEPA. 2002a. Consolidated Assessment and Listing Methodology: Toward a Compendium of Best Practices. US Environmental Protection Agency, Office of Wetlands, Oceans, and Watersheds. <http://www.epa.gov/owow /monitoring / calm.html>.

USEPA. 2002b. Methods for measuring acute toxicity of effluent and receiving waters for fresh water and marine organisms, 5th Oct 2002. United States environmental protection agency, office of water (4303T), Washington DC, EPA 821-R02-012. 275.

Vijayavel, K and Balasubramanian, M.P. 2006. Changes in oxygen consumption and respiratory enzymes as stress indicators in an estuarine edible crab, *Scylla serrata*, exposed to naphthalene. *Chemos*., 63: 1523-1531.

Zielinski, S and Portner H.O. 2000. Oxidative stress and antioxidative defense in cephalopods: A function of metabolic rate or age? *Comp. Biochem. Physiol. Part B*. 125: 147-160.

****